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Results of a four years sweat test analysis for screening of Cystic Fibrosis in the Piedmont region of Italy

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Purpose of this work is to check whether significant differences exist among sweat tests of children attending Cystic Fibrosis (CF) Centre at different step of CF neonatal screening, in the period from December 2000 to October 2004.
137384 newborns were screened whit assay for immunoreactive trypsinogen (IRT).
2298 (1.7%) had IRT higher than cut off and were submitted to genetic analysis.
265 of these have finalized the screening programme to sweat test. 40 (15.1%) were positive (chlorine > 40 meq/l) and 225 (84.9%) negative (chlorine < 40 meq/l).
Chlorine concentration was determinate with sweat test (Gibson & Cook method for ionic chromatography).
265 children submitted to sweat test were subdivided into 3 different groups depending on the 3 different screening's steps in which they arrived at sweat test.
First group consists of children with IRT < 80 ng/ml and 1 mutation; second group consists of subjects with a firts IRT > 80 ng/ml, without mutations and with second IRT higher than 40 ng/ml and third group consists of children with IRT > 80 and 1 mutation.
Following table compares results of sweat tests (chlorine concentration expressed as medium value) in the 3 different groups for positive and negative subjects.

		positive	negative	total
1 group	Total Number (%):	8 (7,8%)	95 (92,2%)	103 (38,9%)
	Chlorine concentration (medium value):	68,377	25,967	
2 group	Total Number (%):	1 (1,1%)	87 (98,9%)	88 (33,2%)
	Chlorine concentration (medium value):	98,711	23,151	
3 group	Total Number (%):	31 (41,9%)	43 (58,1%)	74 (27,9%)
	Chlorine concentration (medium value):	114,432	26,425	
total		40	225	265

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Neonatal screening's utility for early diagnosis of Cystic Fibrosis in the Piedmont region of Italy

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Purpose of this work is to confirm the benefit of a four years neonatal screening's program for early diagnosis of Cystic Fibrosis (CF) in the Piedmont regions of Italy, in the period from December 2000 to October 2004.
The neonatal screening program is a two step protocol which combines the assay for immunoreactive trypsinogen (IRT) with the analysis of 31 mutations in the CFTR gene using the OLA-PCR-SCS method directly on the Guthrie blood spot.
In 47 month period, 137384 newborns were screened. The results of our screening strategy is: 2298 (1.7%) had IRT higher than cut off and were analysed with the PRC-OLA. 26 newborns with 2 mutations in the CFTR gene were directly referred to the CF Centre. The sweat test was performed on 202 heterozygous infants: 17 were diagnosed as CF. Between 43 newborns diagnosed as CF (IRT> cut off, sweat test positive), 26 presented two mutations, 16 one mutation and 1 no detectable mutations. The 17 patients who had none or only one mutation were analysed with DHPLC:12 patients carried two mutations and 3 patients only one mutation.
Finally, 36.6% of patients are homozygotes (93.3% ΔF508, 6.7%R117H), 46.3% compound heterozygotes and 9.8% heterozygotes. Incidence of CF is approximately 1/3195 (0.03%) and carrier frequency 1/12 (8.0%).
Four false negative patients were ascertained (detection rate: 91.5%).
Consequently, the screening programme permits an early and accurate diagnosis of CF, diminishing average time from 18 month, without neonatal screening, to 6 week.

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Age appropriate reference intervals for sweat sodium and sweat chloride in the diagnosis of cystic fibrosis

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Aims: To establish age appropriate reference intervals for sweat sodium and sweat chloride concentrations measured by quantitative pilocarpine iontophoresis (QPIT) at Alberta Children's Hospital.
Methods: A retrospective chart review of consecutive patients with QPIT performed in the years 1996-2000 (n=1099) was undertaken. Alberta Children's Hospital is a centralized sweat testing center for southern Alberta, Canada. Patient demographics, sweat sodium concentration, sweat chloride concentration and definitive diagnosis data were extracted from the charts. Sweat sodium was determined by flame photometry (IL943) and sweat chloride was determined by chloridometer (Radiometer CMT 10). Age dependent reference intervals for sweat sodium and chlorides were determined parametrically (95% confidence interval).
Results: The population of 1099 patients tested included 29 patients with clinically confirmed cystic fibrosis that were removed from the dataset prior to calculating the reference intervals.

Age	Sample Size	Sweat Chloride Reference Interval	Sweat Sodium Reference Interval
0-365 days	242	3-20 mmol/L	5-27 mmol/L
1y – 5y	343	4-22 mmol/L	6-36 mmol/L
>5y	173	6-56 mmol/L	9-67 mmol/L

Conclusions: Age appropriate reference intervals for sweat sodium and sweat chloride are significantly distinct from the commonly used reference intervals. Future studies will evaluate the clinical impact of applying age specific reference intervals.

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MULTI-CODE® CFTR Gene Multi-Mutation Analysis on Luminex™ Microspheres

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Fifteen years after the characterisation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, and the identification of over 1300 mutations, CF is now to become the first disease targeted for population-wide genetic screening.
MULTI-CODE® CFTR Gene Multi-Mutation Analysis on Luminex™ Microspheres is a high throughput multiplex genotyping of CFTR mutations. It has been developed to screen for the twenty-five most common mutations recommended by The American College of Medical Genetics (ACMG) and for four polymorphisms.
The system begins with multiplex PCR with coded primers and incorporation of a labelled AEGIS™ (An expanded Genetic Information System) triphosphate which generates a target specific signal. Each specific product is then captured at room temperature using an ERA-CODE® system which allows molecular recognition with an ERA-CODE sequence covalently fixed to a single microsphere. Automated flow cytometry with the Luminex100 allows each microsphere to be identified and tested for associated reporter signal.
The Luminex technology allows up to 100 different beads in a single tube, each of them being identified by a specific red-orange fluorescence. A normal or mutated allele will be detected by a green fluorescence.
The test is rapid (4 hours from the DNA to the result), is performed in a single tube from the PCR to the detection without any manipulation of the samples. Using 96-wells microplates, it can be adapted to automation and allows large series to be performed.
This test was evaluated on previously genotyped samples carrying the mutations and on 500 blood spots of newborns. This technique distinguishes between homozygotes and heterozygotes for all the mutations. The results were in agreement with data obtained using DHPLC or ARMS technology.